FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
DEBE 1 PCT SEQ/dln	**09/914220**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/00506	**18 FEB 2000 (18.02.00)**	**22 FEB 1999 (22.02.99)**

TITLE OF INVENTION
DNA SEQUENCE OF A PROTEIN THAT IS SIMILAR TO FKBP

APPLICANT(S) FOR DO/EO/US
Burkhard SCHULZ

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

PTO 1449
Statement under 37 CFR 1.821(f)
Sequence Listing - paper and diskette

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
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21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1,000.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	**$860.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT = **$860.00**

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). **$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	13 - 20 =	0	x $18.00	**$0.00**
Independent claims	2 - 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**

TOTAL OF ABOVE CALCULATIONS = **$990.00**

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐ **$0.00**

SUBTOTAL = **$990.00**

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + **$0.00**

TOTAL NATIONAL FEE = **$990.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐ **$0.00**

TOTAL FEES ENCLOSED = **$990.00**

Amount to be: refunded	$
charged	$

☒ A check in the amount of **$990.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **8-3220** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

G. Patrick Sage
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G. Patrick Sage
SIGNATURE

G. Patrick Sage
NAME

37,710
REGISTRATION NUMBER

August 14, 2001
DATE

* * * * *

Applicant : Burkhard SCHULZ

Title : DNA SEQUENCE OF A PROTEIN THAT IS SIMILAR TO FKBP

* * * * *

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

A soon as a Serial Number and Filing Date have been accorded the above-identified national phase application, kindly amend as follows:

IN THE ABSTRACT: Kindly replace the Abstract with the Abstract provided herewith.

IN THE CLAIMS: Kindly cancel all of the claims, 1 through 13, and replace by Claims 14 through 26 as provided herewith.

R E M A R K S

The present application is a national phase filing of PCT/DE00/00506 of 18 FEB 2000.

Applicant has cancelled all of the originally-filed Claims. New Claims 14 through 26 have been added to better encompass the full scope and breadth of the invention notwithstanding Applicant's belief that the Claims would have been

allowable as originally filed. Accordingly, Applicant asserts that no Claims have been narrowed within the meaning of *Festo*.

A new Abstract is provided herewith.

A PTO 1449 listing references cited in the PCT application and the priority German application is enclosed for the Examiner's convenience.

Entry of the new Claims and Abstract and early and favorable action on the merits of this application are respectfully solicited.

Respectfully submitted,

THE FIRM OF HUESCHEN AND SAGE

By: G. Patrick Sage
G. PATRICK SAGE

Dated: August 22, 2001
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Enclosure: Postal Card Receipt
Claims 14 through 26
Abstract
PTO 1449 - two pages

DEBE1PCTSEQ/dln

ABSTRACT

The present invention refers to a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7, a fragment or derivative thereof, and a nucleic acid sequence that hybridizes with a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7, the sequence having the biological activity of a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7. The invention further pertains to transgenic plants and their seeds comprising a recombinant nucleic acid sequence of the present invention.

CLAIMS

14. A nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7, a fragment thereof, a derivative thereof, and a nucleic acid sequence that hybridizes with a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7, the nucleic acid sequence having the biological activity of a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7.

15. The nucleic acid sequence of claim 14, wherein the hybridizing nucleic acid sequence hybridizes under stringent conditions with a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7.

16. A polypeptide comprising an amino acid sequence selected from SEQ ID NO:3, SEQ ID NO:6 and SEQ ID NO:8.

17. A vector comprising a nucleic acid sequence of claim 14.

18. The vector of claim 17, further comprising one or more regulatory elements that ensure the transcription and/or translation of the nucleic acid sequence of claim 14.

19. A method for the production of plants, comprising the stable integration of at least one nucleic acid sequence of claim 14 into the genome of plant cells or plant tissues and the regeneration of these modified plant cells or plant tissues into plants.

20. The method of claim 19, wherein the integrated nucleic acid sequence further comprises one or more regulatory elements which ensure the transcription and/or translation of the nucleic acid sequence.

21. The method of claim 19, wherein the integrated nucleic acid sequence is expressed in antisense orientation.

22. The method of claim 19, wherein the integrated nucleic acid sequence has the activity of a ribozym which represses the biological activity of the endogenous nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:7.

23. The method of claim 19, wherein the nucleic acid sequence is integrated via homologous recombination into the genomic region of the homologous endogenous gene.

24. A transformed plant cell or transformed plant tissue, comprising a stable integrated nucleic acid sequence of claim 14 in the genome of the plant cell or plant tissue.

25. The plant cell or plant tissue according to claim 24, regenerable to a seed producing plant.

26. A transgenic plant and its seeds comprising a recombinant nucleic acid sequence according to claim 14.

Claims

1. Nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 or their fragment or derivative or a nucleic acid sequence that hybridizes with the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 and having the biological activity of the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7.

2. Nucleic acid sequence according to claim 1, whereas the hybridising nucleic acid sequence hybridises under stringent conditions with the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7.

3. Polypeptide, comprising an amino acid sequence according to SEQ ID NO:3, SEQ ID NO:6 or SEQ ID NO:8.

4. Vector, comprising a nucleic acid sequence according to claim 1 or 2.

5. Vector according to claim 4, further comprising one or more regulatory elements that ensure the transcription and/or translation of the nucleic acid sequence according to claim 1 or 2.

6. Method for the production of plants, comprising the stable integration of at least one nucleic acid sequence according to claim 1 or 2 into the genome of plant cells or plant tissues and regeneration of the obtained plant cells or plant tissues to plants.

7. Method according to claim 6, whereas the integrated nucleic acid sequence further comprises one or more regulatory elements, which ensure the transcription and/or translation of the nucleic acid sequence.

8. Method according to claim 6 or 7, whereas the integrated nucleic acid sequence is expressed in antisense orientation.

9. Method according to claim 6 or 7, whereas the integrated nucleic acid sequence has the activity of a ribozyme, which represses the biological activity of the endogenous nucleic acid sequence according to claim 1 or 2.

10. Method according to claim 6, whereas the nucleic acid sequence is integrated via homologous recombination into the genomic region of the homologous endogenous gene.

11. Transformed plant cell or transformed plant tissue, comprising one stable integrated nucleic acid sequence according to claim 1 or 2 in the genome of said plant cell or plant tissue.

12. Plant cell or plant tissue according to claim 11, regenerable to a seed producing plant.

13. Transgenic plant and their seeds comprising a recombinant nucleic acid sequence according to claim 1 or 2.

Abstract

The present invention refers to a nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 or its fragment or derivative or a nucleic acid sequence, which hybridizes with the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 and having the biological activity of the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 5 or SEQ ID NO:7. The invention further refers to transgenic plants and their seeds comprising a recombinant nucleic acid sequence according to the present invention.

4/pRTS

DNA sequence of a protein that is similar to FKBP

The present invention refers to a nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 or its fragment or derivative or a nucleic acid sequence, which hybridizes with the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 and having the biological activity of the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 5 or SEQ ID NO:7. The invention further refers to transgenic plants and their seeds comprising a recombinant nucleic acid sequence according to the present invention.

Plants sessile way of life requires high adaptability to their habitats environmental factors. The endogenous growth and developmental programs must be attuned to exogenous factors. This presupposes the perception of exogenous factors which are vital for plants survival. Since the site of perception usually differs from the site of response to a stimulus, intercellular as well as intracellular signal transduction must take place. Although stimuli are perceived through different receptors in plants and animals and lead to various responses to stimuli, they often employ the same principles for mediating signals. G-proteins, calcium or calmodulin, respectively, protein kinases and protein phosphatases are elements of signal transduction chains that take place in plants and animals. The general mechanisms of signal transduction are conserved in many cases.

A large family of conserved proteins whose functions in signal transduction is still not well-known are the immunophilins (Schreiber, 1991, Science 251: 283-287). Immunophilins represent a super family whose members can be found in bacteria, yeast, plants and animals. The are located in different cell compartments and participate in highly differing processes of signal transduction. They have been identified as intracellular receptors for immunosuppressive substances in mammalian cells (Handschumacher et al., 1984, Science 266: 544-547). Immunophilins can be subdivided into three classes both structurally and through their binding ability to immunosuppressiva: cyclophilines that bind CyclosporinA, FK506 binding proteins that bind FK506 or rapamycin and parvulines with no

affinity to immunosuppressive substances. CyclosporinA, FK506 and rapamycin are substances that are synthesized by soil-borne fungi. Their effect in mammals is the suppression of the immune response used in transplantation medicine to reduce the rejection of the foreign organ.

The FK506 binding proteins (FKBPs) are subdivided according to their size. The smallest FKBP in eukaryotes, FKBP12, is a relatively well-researched immunophilin. It mediates various answers depending upon the bound immunosuppressive substance (Bram et al., 1993, Mol. Cell. Biol. 13: 4760-4769; Brown et al., 1994, Nature 369: 756-758; Liu et al., 1991, Cell 66: 807-815). The binding of FK506 to FKBP12 leads to complex formation with the calcium/calmodulin dependent protein phosphatase calcineurin.

Calcineurin participates in numerous signal transductions and its inhibition through the FKBP12-FK506 complex mediates, for example, the suppression of T-cell activation. The suppression of the immune response through FK506 is mediated differently by rapamycin namely through the inactivation of calcineurin (Schreiber and Crabtree, 1992, Immunology Today 13:136-142). In a complex with rapamycin, FKBP12 interacts with the protein mTOR (mammalian target of rapamycin). One of the mTOR protein domains shows sequence homology to the catalytic domain of phosphatidylinositol-kinases (Sabatini et al., 1994, Cell 78: 35-43). The answer FKBP12 mediates with rapamycin leads to an arrest in the G1 phase of the cell cycle in interleukin-2 stimulated T-cells. In the absence of immunosuppressive substances FKBP12 interacts with elements from other signal transductions, for instance with the TGF-b receptor (receptor for the transforming growth factor-b) and modulates its function in cell cycle control (Wang et al., 1994, Science 265: 674-676). FKBP12 also participates in the regulation of two intracellular calcium channels, namely the inositol-1,4,5-triphosphate receptor and the ryanodin receptor (Brillantes et al., 1994; Cameron et al., 1995, Cell 83: 463-472). FK506 or rapamycin lead to dissociation from FKBP12 and calcineurin from the calcium channel complexes and thus to an increased calcium-efflux through these channels. The regulation of the calcium channels through FKBP12 was confirmed through examinations of a transgenic mouse mutant, that does not express a functional FKBP12 (Shou et al., 1998, Nature 391: 489-492). FKBP12 deficient mice die before or shortly after birth of a myocardial insufficiency, which was also observed in patients treated with FK506. The calcium conductivity of the ryanodin receptors in the skeletal muscles of these mice

resembles that of the purified receptors with no bound FKBP12.

FKBP59 from mammals was identified as an essential component of steroid receptor complexes which are not bound to ligands (Sanchez et al., 1990, Biochemistry 29: 5145-5152). In this multi-protein complex two heat shock proteins, Hsp70 and Hsp90 were also identified. The binding of FKBP59 to the steroid receptor takes place indirectly via Hsp90 (Peattie et al., 1992). The interaction of FKBP59 and Hsp90 is mediated by the conserved protein-protein interaction motif, the so-called tetratricopeptide repeats (TPR). The TPR motif is a 34 amino acid sequence originally found in proteins participating in cell cycle regulation, transcription regulation, protein transport and heat shock response (Goebl and Yanagida, 1991, TIBS 16: 173-177). The type III TPR domain consists of the triple repetition of the TPR motif, whereas two of the repetitions directly follow each other. The distance to the first TPR motif is conserved and has 10-16 amino acids. The sequence motif forms amphipatic α-helices, named "knob-hole" structures and can mediate a specific protein-protein interaction.

The binding of a steroid hormone to the receptor complex leads to the dissociation of FKBP59 and Hsp90. The ligand-bound steroid receptor can now reach the nucleus and is bound to DNA participating in the building of a transcription complex. It is being discussed that FKBP59 and the Hsp proteins are necessary for the conservation of the conformation of the non-ligand-bound steroid receptor (Pratt and Welsh, 1994, Sem. Cell Biol. 5: 83-93).

A few years ago immunophilins from plant extracts from *Vicia faba* were isolated via their affinity to FK506 and cyclosporinA (Luan et al., 1994, Proc. Natl. Acad. Sci. USA 91: 984-988). During this process an FKBP12 was isolated, which showed high sequence homology to FKBP12 from yeast and animals (between 47%-51% amino acid sequence identity). *In vitro* this FKBP12 from *Vicia faba* showed, however, little affinity to calcineurin, and expressed in yeast it did not mediate the effect of FK506 and rapamycin (Xu et al., 1998, Plant J. 15: 511-519). In *Vicia faba* injected FK506 could only inhibit a calcium dependent regulation of calium channels in guard cells, if human FKBP12 was also applied at the same time (Luan et al., 1993, Proc. Natl. Acad. Sci. USA 90: 2202-2206), which is a hint to the presence of a FKBP12-FK506 signal transduction chain in plant cells, without having an endogenous receptor for FK506.

In plant breeding, it has been attempted to improve desired characteristics of crops and ornamental plants for a long time. Until now these improvements have been achieved with very long-term and costly methods of conventional breeding. The development of new plant varieties and products often takes 10 to 15 years. An alternative strategy is to provide certain plants with the characteristics desired for improvement available through the use of genetic information such as „marker assisted breeding" and genetically engineered alterations. A desired aspect in this connection is increasing yield by enlarging the number or volume of seeds that can be harvested (1000 grain weight), which are the yield-determining organs of many crops. In this case, however, the desired goal is not just the enlargement of the parameters number and volume, but also avoiding loss of seeds through seed shed before harvest and the reduction of threshing loss during harvest. During seed maturation, after filling of the seed with storage compounds, seed domance will be initiated. This developmental phase is characterized by drying of the seed-carrying organs, for instance, siliques and other opening fruits. The siliques burst during this phase along the seam of the organ in order to spread the seeds. From the point of view of production, this important process for the spread of a plants generative organs is not desired. Seeds freed from the silique before the beginning of the harvest through weather conditions such as precipitation or wind, as well as seeds which fall to the ground through the mechanical manipulations during the harvesting process must be counted as harvest loss.

The change in the entire architecture of a plant with the goal of reducing the stem growth is for crops, whose yield is determined through reproductive organs has been a goal of breeding for quite some time. On one hand, this would make possible the shift of the relationships of biomass from vegetative areas of the plant that are not relevant to the yield in yield-determining harvest organs. On the other, shortening the stem sections would increase the rigidity of the plants against weather influences. These aspects are particularly important in breeding grain plants since a relevant part of harvest loss is caused by lodging before harvest. The yield of grains has been greatly increased over the past 50 years while dwarf mediating mutations such as Rht1, Rht2, Rht3 in wheat or D8 and D9 in maize were crossed in commercially used varieties and participating significantly in the increase of the yield. The result of this breeding was lines that did not react with an elongation of the stem but increased the seed yield when artificial fertilizer was added (Silverstone and Sun, Trends in Plant Science 5: 1-2 (2000).

Reduced growth is also often desired in ornamental plants. This is especially necessary for the creation of bonsai plants as well as miniature versions of many ornamental plants and cut flowers – sunflowers, for instance. In this context, twisted growth could, again, be of interest since shrubs and trees with twisted growth can be found in corkscrew willows, ficus and in other ornamental plants on the market. In the production of timber twisted growth of stems and branches can be a desired characteristic. In trees, the production of so-called "compression wood" can be gained with changed rigidity characteristics and changed yields. Here, increased amounts of compression wood is made in highly lignified stems by changes of the normal growth direction to reduce mechanical stress. This characteristic can be used for the production of wood for making paper. Wood, used for building or for the production of furniture can be produced by twisted growth of the harvestable stem sections with changed rigidity because this changes the compression strength and tensile strength of the stem sections. Fiber producing plants can produce new, desired characteristics for processing and physical characteristics (rigidity etc.) through twisted grown plant fibers.

Said problems are solved by the embodiments characterized in the claims.

The invention is explained in more detail with the following illustrations.

Figure 1 shows the genomic sequence of the twisted dwarf gene from *Arabidopsis thaliana* type Wassilewskija including the promoter area. The start and stop codons are underlined. Exon sequences are marked in bold, intron sequences are in italics. At the beginning of the line of nucleotide sequences the positions are numbered. In the lines beneath the nucleotide sequence, each amino acid sequence of the open reading frame is named. The amino acid position are numbered at the end of the line.

Figure 2 shows an illustration of the amino acid alignment of the twd gene from *Arabidopsis thaliana* (TWD) and *Lycopersicon esculentum* (TTP). Identical amino acids are connected by a vertical line, similar amino acids are linked by two points.

Figure 3 shows an illustration of the amino acid alignment of the *twd* gene from *Arabidopsis thaliana*

(TWD) and *Zea mays* (ZmTWD). Identical amino acids are connected by a vertical line, similar amino acids are linked by two points.

The term "vector" as used herein, refers to naturally occurring or artificially created constructs for the uptake, multiplication, expression or transfer of nucleic acids, for instance, plasmids, phagemids, cosmids, artificial chromosomes, bacteriophages, viruses, retroviruses.

The term "derivate" as used herein, refers to nucleic acid or amino acid having one or more deletions, substitutions, insertions and/or inversions.

The term "fragments" as used herein, refers to nucleic acid sequences or amino acid sequences comprising a part of the nucleic acid sequences or amino acid sequences according to the invention.

The term "transformed plant cells" as used herein, refers to plant cells and plants or plant organs derived therefrom, that were genetically altered by the transfer of nucleic acids, for instance, plasmids, phagemids, cosmids, artificial chromosomes, bacteriophages, viruses, retroviruses or nucleic acid sequences not inserted in vector constructs.

The term "regulatory element" as used herein, refers to nucleic acid sequences for regulating the expression of a gene. These nucleic acid sequences include promotor areas of a gene as well as regulatory areas within the translated as well as non-translated regions of a gene.

The term „hybridisation" or „hybridising" as used herein, means stringent and less stringent conditions; see. Sambrook et al., Molecular Cloning, Cold Spring Harbour Laboratory (1989), ISBN 0-87969-309-6. An example of stringent hybridisation conditions is: hybridisation in 4 x SSC at 65° C (alternative in 50% Formamid and 4 X SSC bei 42° C), followed by several washing steps in 0,1 x SSC at 65° C for altogether one hour. An example for less stringent hybridisation conditions is hybridisation in 4 x SSC at 37° C, followed by several washing steps in 1 x SSC at room temperature.

The term "homologous sequence" or "homolog" as used herein, refers to a nucleic acid or protein

sequence that shows the activity of the nucleic acid or protein sequences according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7. Nucleic acid sequences hybridizing with the sequences according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 or parts of this sequence under stringent or less stringent conditions are also homologous sequences. Further, homologous sequences are also nucleic acid or protein sequences or parts of these, which are significantly similar to nucleic acid and amino acid sequences in this inventions, determined by the similarity algorhythm BLAST (Basic Local Alignment Search Tool, Altschul et al., Journal of Molecular Biology 215, 403-410 (1990) (Matrix: Blosum 62, Gap existence cost: 11, Per residue gap cost:1). Sequences considered to be significantly similar, as used herein, are those that show, for instance, a signification niveau (probability) of $P < 1e^{-30}$ within the use of standard paramaters in the Blast service of NCBI if they can be compared to the sequences according to SEQ ID NO:1 or SEQ ID NO:2 or parts of these.

The term „marker assisted breeding" as used herein, refers to the selection of plants employing genetic information and molecular markers derived from this such as AFLP, RFLP, SNP etc in breeding programs. The above mentioned markers represent all types of nucleic acid sequence changes that can be proven and used for the screening of plant populations through diagnostic DNA analyses such as PCR, restriction analysis or hybridisation.

From a population of *Arabidopsis thaliana* plants that were mutagenized through T-DNA insertion, a mutant could be isolated that is characterised by a drastic change of its phenotype. The twisted dwarf mutant (hereafter *twd*) has a pleiotropic phenotype which is manifested in plant architecture and physiology. The *twd* mutant is greatly reduced in its total height, at the time of its senescence it only attains one third total height of the wild type (approx. 25 cm). The mutant is dark green like other Arabidopsis dwarf mutants and seems compact due to its shortened inflorescence. The growth of the rosette leaves is characterised through extreme epinastic bending and an irregular surface. On the spread rosette leaves it can be recognized that the ratio of leaf length to leaf width is smaller than in wild type rosette leaves. The greatly shortened main stem of the inflorescence has a greater diameter than wild-type plants. The disoriented growth of the stem makes the mutant look unusual for *Arabidopsis thaliana* and is reminiscent of a cirrus plant. The disoriented growth of the plant organ can also be observed in the anthers and carpels.

The DNA sequences of the mutated gene were isolated from the mutant through the plasmid rescue method in *E.coli*. Here, the fact is used that the T-DNA used for mutagenesis contains two sequence regions that make replication and selection in *E.coli* cells possible. Through restriction of genomic DNA of the twd mutant with suited restriction endonucleases (here *Eco*RI), DNA fragments were created, which were introduced after self-ligation in transformation competent *E.coli* cells. The selection of plasmid-bearing clones is attained through resistance against the antibiotic ampicillin on solidified culture media. DNA of the transformed plasmids was isolated from these clones and identified by restriction with restriction endonucleases and subsequent hybridisation with hybridisation probes. Clones were identified that contained apart from the used T-DNA sequences also DNA sequences from the mutated twd locus. These DNA sequences were isolated and subcloned into the vector pBluescript(SK-)® (Stratagene). The inserted DNA sequences were sequenced with the chain termination method according to Sanger. For the subsequent isolation of genomic and cDNA clones of the twd gene, the cloned DNA sequences were used as hybridisation probes.

The present invention refers to a nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 or its fragment or derivative or a nucleic acid sequence hybridising with the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 having the biological activity of the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7. Furthermore, the invention refers to a nucleic acid sequence hybridising under stringent conditions to the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7. The nucleic acid sequence according to SEQ ID NO:1 represents the genomic DNA sequence, the nucleic acid sequence according to SEQ ID NO:2 represents the cDNA sequence of the twisted dwarf gene from *Arabidopsis thaliana*, SEQ ID NO:5 represents a fragment of the cDNA sequence of the homolog twisted dwarf gene from *Lycopersicon esculentum*, SEQ ID NO:7 represents a fragment of the cDNA sequence of the homolog twisted dwarf gene from *Zea mays*.

Furthermore, the invention refers to a polypeptid comprising an amino acid sequence according to SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:8. The amino acid sequence can be modified so that the

amino acid sequence shows amino acid additions, deletions or insertions at one or more positions. The amino acid sequence according to SEQ ID NO:3 represents the amino acid sequence of the twisted dwarf protein from *Arabidopsis thaliana*, SEQ ID NO:6 represents the amino acid sequence of the homolog twisted dwarf protein from *Lycopersicon esculentum*, SEQ ID NO:8 represents the amino acid sequence of the homolog twisted dwarf protein from *Zea mays*.

The present invention refers to nucleic acid sequences from a plant genome, particularly preferred from *Arabidopsis thaliana*, *Zea mays* or *Lycopersicon esculentum* that contain the coding region of an FKBP-like (FK506 binding protein) gene (twisted dwarf), whose activity controls the shaping of the entire architecture of the plant, in particular cell growth, growth orientation, degree of branching, etc. The discontinuation of these activities, for instance due to mutation or deletion in a plant genome leads to a change in the entire architecture of the plant through reduction of cell growth, disorientation of the growth of all organs above and below ground, reduction of branching of the stem, changes in the reaction towards brassinosteroids and their precursors and derivatives and the change in the reaction of the roots to gravitropism resulting in the change of ethylen production and ethylene induced signal transmission. The nucleic acid sequence according to the invention can be inserted into a vector, which also comprises one or more regulatory elements that control the transcription and/or translation of the nucleic acid sequence according to the invention. Further, the invention refers to vectors, for instance plasmids, and host cells, such as yeasts and bacteria including the nucleic acid sequence according to the invention.

The invention also refers to the use of the nucleic acid sequences according to the invention for the identification and isolation of homologs or related genes from other dicotyledoneus and monocotyledoneus plants similar to FKBP-like genes through data bank alignments, hybridisation or with PCR techniques known to the person skilled in the art.

In order to find homologs or related FKBP-like genes from other plants by means of a data bank alignment the nucleic acid sequences according to the invention or the polypeptid sequences according to the invention derived therefrom can be employed in data bank alignments with the similarity logarithm BLAST (Basic Local Alignment Search Tool, Altschul et al., Journal of Molecular Biology

215, 403-410 (1990), blastn for alignments with nucleic aced sequences, tblastn for alignments with polypeptid sequences) by using standard parameters in the Blast service of the NCBI. In this connection gene sequences with a significance level of $P < 1e^{-30}$ which show also a similar domain structure as the nucleic sequences according to the invention or the polypeptid sequences derived therefrom are referred to as homologs or related to the twd gene.

The present invention further refers to a method for the production of plants comprising the stable integration of at least one nucleic acid sequence according to the invention into the genome of plant cells or plant tissues and regeneration of the obtained plant cells or plant tissues to plants. Particularly, the invention refers to a method in which the integrated nucleic acid sequence also comprises one or more regulatory elements, that ensure the transcription and/or translation of the nucleic acid sequence. Particularly preferred is a method in which the integrated nucleic acid sequence is expressed in antisense orientation. Further particularly preferred the invention refers to a method in which the integrated nucleic acid sequence has the activity of a ribozyme that represses the biological activity of the endogenous nucleic acid sequence encoding a FKBP-like protein. The nucleic acid sequence according to the invention and optionally its regulatory elements can be integrated through homologous recombination into the genomic DNA of target cells. The homologous recombination can also be executed so that the nucleic acid sequence according to the invention is integrated into the genomic area of the endogenous gene, wich encodes a FKBP-like protein.

The method according to the invention is not limited to a certain plant variety, but can be applied in all plants. Preferred plants are, for instance, crops and ornamentals, e.g. grains such as wheat, maize, rice, rye or barley; legumes such as peas, beans, chickpeas, lentils or soybeans; brassicaceen such as rape or mustard; fibrous plants such as flax, hemp or cotton; trees such as firs, poplar, beech, oak or nut trees; ornamental brushes; or solanaceae such as tomato or potato.

The invention further refers to transformed plant cells or transformed plant tissue comprising a nucleic acid sequence according to the invention that is stably integrated into the genome of the plant cell or the plant tissue. Preferred are transformed plant cells or transformed plant tissue, which can be regenerated into a seed producing plant. The invention further refers to transgenic plants and their seeds comprising

a recombinant nucleic acid sequence according to the invention.

The invention further refers to mutants, for instance, in *Arabidopsis thaliana*, *Zea mays* or *Lycopersicon esculentum* in which the endogenous twisted dwarf DNA sequence (FKBP-like (FK506 binding protein) gene) is changed e.g. either through T-DNA insertion or through deletion or insertion of DNA of various size and in which the plants show the above mentioned phenotypical changes. Also, the invention refers to transgenic plants in which mutated DNA sequences in the above mentioned genetic sequence are phenotypically restored to wild-type through the introduction of intact gene copies.

The nucleic acid sequence or the method according to the invention can be used to produce transgenic plants with changed, meaning, disoriented growth. Disoriented growth is manifested in a change in the structure of cell walls and intracellular support and frame elements (for instance, cytoskeleton). Such changes can be employed in the production of plants used in the production of fibers and other materials with new, changed material characteristics. Through the twisted growth of lignified support organs, for instance, in trees wood can be acquired on one hand through the formation of so-called "compression wood" with changed rigidity characteristics and perhaps changed yields. Fibers producing plants can produce plant fibers with new, desired characteristics of processing and physical characteristics (rigidity etc.) through twisted growth.

Another aspect of twisted growth applies to the twisting of growth direction along the longitudinal axis e.g. siliques, from crops. This growth results in a reduced spontaneous burst of the siliques at the point of seed ripening. At seed ripening, after the seeds are filled with storage compounds, the phase of seed domancy begins. This phase is characterized by the drying and bursting of the siliques to spread of seeds. Twisted growth of the silique impedes the silique from fully opening and thus leads to reduced seed shed. Thus, harvesting losses in the mechanical manipulation during the harvesting process and harvest loss through unwanted premature seed shed are reduced. These characteristics are especially useful in all crops with harvestable siliques, e.g. soy, rape, mustard or legumes of all kinds.

It could also be shown that the nucleic acid sequence according to the invention is not only responsible

for growth direction, but also for the size of the plant itself. Thus, the present invention refers also to transgenic plants with a smaller habitus compared to their wild-type plants. This is valid for all grains such as wheat, maize, rice, rye, barley, etc. that have on the one hand a higher stability during growth and yield due the reduction of stem length and thus less stem breakage because of, for instance, rain and wind and on the other hand with a higher production of biomass in the harvestable organs. Decreased growth is also often desired in ornamental plants. The production of bonsai plants as well as miniature versions of many ornamental plants and cut flowers, e.g. sunflowers are referred to here in particular. In this context twisted growth can also be of interest since bushes and trees with twisted growth, e.g. corkscrew willows and ficus can also be found in ornamental plants on the market.

The silique as well as the flower is less reduced in its entire length as the other organs, but the stalk, however, is very much shortened. The seeds of the twised dwarf mutants, surprisingly, do not show the strong reduction in size of the other plant organs. Compared to wild type seeds, the seeds of twisted dwarf mutants have a larger volume. Thus, the present invention further refers to an increase of the total weight of the yield of seed bearing plants. Thus, it could be shown that the average number of seeds per silique in twd mutants of *Arabidopsis thaliana* (20) is reduced to approx. one third of the number of a corresponding wild-type plant (57). The number of siliques on one plant was, however, markedly increased in twd mutants (417) compared to wild types (136). This results in an approx. 10% higher yield for the average total amount of harvested seeds per plant for twd mutants. The dimensions of the seeds also show great differences in twd mutants and corresponding wild-type plants. Estimates of the seed volume according to Leon-Klosterziel et al., Plant Cell 6: 385-392 (1994) show an approx. 50% larger volume for seeds from twd plant. These characteristics are particularly useful in all crops with harvestable siliques such as soy, rape, mustard, or all types of legumes.

Independent from the variously characterized reduction in size of single plant organs the irregular and disoriented growth in all plant organs including the root of *Arabidopsis thaliana* has been observed. Apart from the changes in growth, the twisted dwarf mutant leads to a slower development of the plant. This is shown in a longer life-cycle of the twisted dwarf mutant compared to the wild type. After approx. 6 weeks of long day conditions (light phase of at least 16 hours) senescence starts in wild-type. The life-cycle of the twisted dwarf mutant is approx. 1 week longer under long day conditions. Under

short day conditions (light phase of maxium 9 hours) it is approx. 3 weeks. It can be observed that the defect in the twd gene causes the mutant's vegetative life phase to be about 5 days longer (approx. 20% longer vegetative phase). This circumstance can be used for the production of plants for which a delay of begin of senescence is desired, e.g. in ornamental plants.

The decreased total length of the twisted dwarf mutant is caused by shortening of the cells. Epidermal cells from primary inflorescence of the wild-type and the twisted dwarf mutant that are peeled with a fine forceps and then dyed with safranin red show shortened cells from the twisted dwarf mutant of approx. 33%. Spraying experiments of twisted dwarf mutants growing on soil with 10^{-7} M of the brassinosteroid brassinolid show an increased longitudinal growth compared to control experiments. But when double mutants from twisted dwarf and the campesterol-reduktase det2, representing a dwarf mutant from *Arabidopsis thaliana,* that can be complemented to the wild-type through the exogenous application of brassinosteroids, were sprayed with 10^{-7} M of brassinolid, no reaction to the application of brassinolid could be ascertained in these plants, which showed extreme dwarf growth. This result then supports the deduction that the twisted dwarf mutant is a plant that takes part in the reception or the signal transduction of the brassinosteroid response in plants. On one hand, the production of such mutants can lead to the targeted production of plants with reduced growth as ornamental plants or other crops. On the other hand, such plants could be used as models for the study of steroid hormone effects.

The obvious participation in brassinosteroid signal reception and signal processing of the twd gene product makes it possible to create plants that can be influenced by changing the twd gene product itself or the amount of the twd gene product via their reaction towards the plant growth substance brassinosteroid and its derivatives in body plan, life-cycle, yield etc. This makes it also possible to create model systems for studies on the mode of action of brassinosteroid and its derivatives in crops, which can lead to the development of specific growth substances and effectors.

The disoriented growth of the twisted dwarf mutant leads to the question of the mutant being able to to exhibit oriented, asymmetrical growth as a reaction to a unidirectional stimulant (tropism). The plant organs above ground reacted like the wild-type with positive phototropism and with negative gravitropism. The root gravitropism of the mutant twisted dwarf and the respective wild-type was

examined with seedlings grown on vertically positioned agar plates. The vertical re-orientation of the plates after 7 days about 90° led to a change in the growth direction: its angle was measured after 5 more days. An angle of curvature between 80°-100° was defined as a gravitropic response (Yamamoto and Yamamoto, 1998, Plant Cell Physiol. 39: 660-664). In seedlings of the twisted dwarf mutant only 27% of the roots showed gravitropic growth. The other 73% showed agravitropic growth. The roots of the wild type seedlings all went through a change in the growth direction of approx. 90°, which is consistent with a positively gravitropic growth. Agravitropic root growth was also observed in the mutants eir1 (Luschnig et al., 1998, Genes Dev. 12: 2175-2187) and aux1 (Maher and Martindale, 1980, Biochem. Genet. 18: 1041-1053). These mutants are insensitive to exogenous applications of ethylene. In order to study the sensitivity towards ethylene of the twisted dwarf mutant, seedlings were incubated under the same conditions as described for the control, except that 10 ppm ethylene was added to the air. First, the twisted dwarf mutant was examined for its phenotype. The increased ethylene concentration caused phenotypical changes including a shortening of the root, an increase of the diameter of the hypokotyl and a reduction of the leaf blade. These changes were observed in both the wild-type and the twisted dwarf mutant. It is noteworthy that the roots of the twisted dwarf mutants grown under increased ethylene concentration, all showed a gravitropic growth consistent with that of the wild-type. The incresased ethylene concentration, however, could revert none of the other characteristics of the twisted dwarf phenotype. Measurement of the angle of curvature showed that all roots of the twisted dwarf mutant grown under 10 ppm ethylen grew gravitropic, yet only 27% of the twisted dwarf roots grown under air showed normal gravitropism. The roots of wild-type seedlings grew gravitropically under both conditions.

In order to investigate if the root gravitropism of the twisted dwarf mutant was corrected by the effect of the phytohormone ethylen, the influence of inhibitors of ethylene biosynthesis and of inhibitors of ethylene response on the root gravitropism of twisted dwarf and wild-type seedlings was studied. The same experiment described above was conducted but with an addition of silver nitrate, an inhibitor of the ethylene effect, in the Arabidopsis medium. A concentration of 1 µM silver nitrate in the growth medium, and 10 ppm ethylene in the growth chamber led to increased agravitropic growth in the roots of the twisted dwarf mutant. This effect was not found in wild-type plants. Amino ethoxyvinylglycin (AVG), which inhibits the endogenous ethylene biosynthesis, led in a concentration of 1 µM to only

12% gravitropic growth in the roots of twisted dwarf mutants. In this experiment 35% of the roots of twisted dwarf mutants grew gravitropically under the control conditions (air). With the addition of 1 µM AVG however, 41% of the roots of wild type plants also showed an agravitropic growth. Ethylene is generally important for root gravitropism, because the retardation of endogenous ethylene synthesis also led to agravitropic behavior in wild-type roots. Mutants of twisted dwarf in *Arabidopsis thaliana* or *Lycopersicon esculentum* and other plants could serve to produce plants and plant organs that produce or accumulate decreased amount of ethylene. This effect can be precisely employed to influence fruit and seed ripening as well as the lengthening and control of the flowering phase of ornamental plants and crops because these processes are controlled by the amount of ethylene in the corresponding organs or plants.

Since gravitropism of the root in twd mutants under normal growth conditions is strongly reduced and is only reconstituted with the exogenous addition of the gaseous phytohormone ethylene, the characterisation of the root gravitropism can be easily regulated through this characteristic. This influences the anchoring of the root in the soil, which affects the rigidity of ground covering plants as well as the anchoring of crops in the substrate in general. The induction of root gravitropism can be introduced through ethylene at any given point of development. This fact can also be used to regulate other developmental processes of the plant with ethylene.

Since twd is a mutant in a FK506 binding protein, these plants can be used as non-animal models for pharmaceutical research of corresponding immunsuppressiva (especially FK506 (Tacrolimus), Rapamycin, Cyclosporin A and other substances with similar effects) as well as processes of signal transduction of the effect of immunsuppressiva. With this, new cellular interactions and modes of action specific to plants, which are not present in this form in animal systems, can be studied. Among other things, models can also be developed with reduced level of FKBPs in which the effect of not only immunsuppressive substances but also genetically modified ligands can be tested for these substances.

The comparison of the deduced amino acid sequence of the open reading frame of the twisted dwarf cDNA in the current sequence databank shows a sequence identity of 30-33% and a sequence similarity of 43-53% to FKBPs from humans, animals and other plants (PILEUP, Genetic Computer Group,

Wisconsin Package Version 9.1-Unix, Sept.1997 (Gap creation penalty: 5; gap extension pealty: 1)). The deduced twisted dwarf peptide shows for the 14 identified amino acid positions for FK506 interaction identical amino acid exchanges in four cases and conserved amino acid exchanges in four more cases. In the c-terminal region of the peptide, a triple repetition of the TPR motif can be found. For this motif, an interaction of Hsp90 with FKBPs has been proven in animal systems (Callebaut et al., 1992, Proc. Natl. Acad. Sci. USA 89: 6270-6274). Plant mutants from this class of protein genes could be developed as non-animal model systems for the study of the effect and signal tranduction of immunophilins.

The following examples serve to explain the invention and are not to be considered as restricting.

I. General Methods

1. Cloning process

For cloning, the phage vector lambda ZipLox and the therefrom derived plasmid PZL-1 (Newman et al., 1994, Plant Phys. 106: 1241-1255) as well as the phagemid pBluescript (pBS) (Short et al., 1988, Nucl. Acids Res. 16: 7583-7600) were employed. For the expression in *E.coli* the expression vector pET3-His (Novagen) was employed. For the transformation of yeasts the vectors pAS1 and pACT2 (Clontech, Matchmaker 2-Hybrid System) and pRS314 (Sikorski and Hieter, 1989, Genetics 122: 19-27) were employed. For plant transformation the gene constructions were cloned in pRT-W NotI (Überlacker and Werr, 1996, Mol. Breeding 2: 293-295) and the binary vector pGPTV-Bar (Becker et al., 1992, Plant Mol Biol. 20: 1195-1197).

2. Bacteria and yeast strains

For the pBluescript KS (pBS) vector, the plasmid pZL-1 as well as for pAS1, pACT2 and pGPTV constructs the *E. coli* strain DH5α (Hanahan et al., 1983, J. Mol. Biol. 166: 557-580) were employed. The expression of the twisted dwarf protein was performed in the *E.coli* strain Bl21 (Studier and Moffat, 1986). The transformation of the pGPTV constructs in Arabidopsis plants was performed with

the help of the *Agrobacterium tumefaciens* strain GV3101:pMP90 (Koncz and Schell, 1990, Mol. Gen. Genet. 204: 383-396). The transformation of 2-hybrid constructs was performed in the yeast strain Y190.

3. Transformation of *Agrobacterium tumefaciens*

The transfer of DNA in Agrobacteria was performed through direct transformation with naked DNA according to Höfgen and Willmitzer (1988, Nucl. Acids Res. 16: 9877). The plasmid DNA transformed into Agrobacteria was isolated with the Birnboim and Doly method (1979, Nucl. Acids Res. 7: 1513-1523) and after suited restriction digest separated with gel electrophoresis to prove the correctness of the inserted DNA.

4. Plant transformation

With a positive colony 150 ml antibiotics containing YEB medium were inoculated and shaken for 2 days at 28°C. With 10-15 ml of this culture 500 ml antibiotics containing YEB-medium were inoculated. This culture was incubated over night at 28°C on the shaker and pelleted the next day for 15 min. at 4,000 rpm. The sedimented bacteria were taken up in infiltration medium. The concentration of the suspension was determined by turbidity measurement and set at a OD600 (optical density) between 0,8 and 1,2. 400 ml beakers filled with Agrobacteria suspension were put into a vakuum exicator. Pots with Arabidopsis plants were placed upside-down on the beakers so that the inflorescences of the plants reached into the Agrobacteria suspension. A vacuum of 10-30 mbar was applied for 15 min and then the vacuum exicator was quickly aired. A bacteria suspension was employed for as many as four continuous infiltrations. Afterwards, the plants were kept further under long-day conditions (16 hours light/8 hours darkness) until the siliques were ripe. The 10 plants in one pot were put in two bags (2 pools) of 5 plants each to collect the seeds when the oldest siliques were ripe. The well-dried seeds could be directly sewn on soil for a selection with BASTA® (Aventis CropScience, S.A., Lyon, Frankreich)

The sowing took place in pools which were separated into large flats. The first spraying of the seedlings with BASTA® solution took place when the cotyledons were fully developed. The seedlings were sprayed 1-2 more times with BASTA® during the following 6 days. Non-BASTA® resistent seedlings bleached at the cotyledon stage and did not further develop. The resistent seedlings grew to seed maturation and the seeds of individual plants were harvested.

Example 1:

Isolation of the FKBP-like twisted dwarf gene from T-DNA tagged insertion lines of *Arabidopsis thaliana* by means of plasmid rescue and the isolation of von full length cDNA and genomic clones from gene libraries

From a *Arabidopsis thaliana* line transformed with T-DNA (Feldmann, 1991, Plant J. 1:71-82; Forsthoefel et al., 1992, Aust. J. Plant Physiol. 19: 353-366) a 200 bp long DNA sequence of the twisted dwarf gene flanking the T-DNA insertion was isolated through plasmid rescue (Schulz et al., 1995, Plant Mol. Biol. Manual, pp 1-17). Radioactive labeled probes produced with the plasmids attained from the plasmid rescue (clone pBUB 52) were employed for screening the CD4-7 lPRL-2 cDNA library (Newman et al., 1994, Plant Phys. 106: 1241-1255) and the CD4-11 genomic cosmid library (Schulz et al., 1995, Plant Mol. Biol. Manual, pp 1-17). Approx. 200 000 clones from the cDNA library were screened with these hybridisation probes. From a positively reacting λ-clone a plasmid was isolated through *in vivo* excision and analyzed by determining of the DNA sequence (dideoxy method: Sanger et al., 1977, Proc. Natl. Acad. Sci. USA 74: 5463-5467). The primary structure of the twisted dwarf protein was deduced from this DNA sequence (clone pBUB 65). The cDNAs were then used to isolate a genomic clone from the cosmid library.

Example 2:

Expression of twisted dwarf peptides in *E.coli*, purification of the proteins and production of antisera against the protein in rabbits

Partial cDNA sequences that code for the amino acid positions 1-324 and 1-187 were amplified with

PCR and ligated after restriction digest with *Bam*HI and *Xho*II in the reading frame of the His tag sequence of the linearised vector pET3 with *Bam*HI. Transformation competent Bl21 cells were transformed with the ligations and the expression of the peptides were proven after induction with IPTG in crude extracts on Laemmli gels. The fusion peptides with the His tag were purified with Ni-NTA agarose (Novagen). The apparent molecular weights were determined after being compared with size markers as 33kDa for the peptide that includes the region from amino acid positions 1-187 and as 44kDa for the peptide that includes the region from amino acid positions 1-324. To immunise rabbits the unpurified peptides (Pos. 1-187) were purified with a preparative SDS-PAGE gel. The protein band were identified by staining the gel with Cu2+ ions, cut out and ground with mortar and pestil. The homogenised gel was resolved in buffer and used to immunize rabbits by the company BioGenes (Berlin). After the first immunisation, two other booster immunisations followed before antiserums against the twisted dwarf protein could be extracted (by bleeding the animals. The recognition of the twisted dwarf protein through the antiserum was tested in immunoblot experiments.

Example 3

Transformation of mutated *Arabidopsis thaliana* plants with a construction for the overexpression of the coding region of the twisted dwarf protein

For the complementation of twisted dwarf mutants (twisted dwarf1-1, twisted dwarf1-3, twisted dwarf1-4) the open reading frame of the twisted dwarf gene was amplified with PCR from the plasmid BUB65 and cloned after *Bam*HI/*Bgl*II restriction digest into the *Bam*HI-site of the vector pRT-Ω NotI. The pRT-Ω NotI contains in front of the *Bam*HI-restriction site a CaMV 35S promoter as well as a Ω sequence from the tobacco mosaic virus, which can increase the translation of various reporter genes in plants 2-10 fold (Gallie et al., 1989, Plant Cell 1: 301-311). A polyadenylation signal from the cauliflower mosaic virus was inserted behind the *Bam*HI restriction sequence. Sequencing the insert showed that the cloned twisted dwarf cDNA sequence underwent no change in sequence. The cassette was excised with the restriction enzyme *Asc*I from the pRT-Ω NotI vector and ligated after fill-in of protruding ends with Klenow polymerase into the *Hin*dIII site of the binary plant vector pGPTV-BAR which was filled-in as well. The uidA reading frame was deleted earlier from the pGPTV-BAR with a

SmaI/EcoRI restriction digest.

The transformation of twisted dwarf mutants with a vacuum infiltration of flowers was performed with the binary vectors using the Agrobacterium strain GV3101 pMP90. Transgenic *Arabidopsis thaliana* plants were selected for herbicide resistance as described above and analysed phenotypically. The presence and the structure of the transformed constructs in the transgenic plants was ascertained with a DNA gel blot analysis. All of the mutant plants transformed with an intact twisted dwarf gene sequence showed a reversion of the phenotype to the wild-type.

Example 4

Sequence analysis of various mutated twisted dwarf alleles from *Arabidopsis thaliana* mutants

Further *Arabidopsis thaliana* mutants which showed the phenotype of twisted dwarf mutant were isolated from various mutagenized populations. With a crossing analysis of the mutants generated by T-DNA insertion it could be shown that the different mutant alleles represented the same gene. In DNA gel blot experiments a restriction fragment length polymorphism (RFLP) could be shown for two mutants.

For an exact analysis of twisted dwarf mutants PCR products of the mutated alleles of the gene were sequenced and compared to the wild-type sequence. The T-DNA insertion in the twisted dwarf allele 1-1 lies in the fifth exon at position +1484. A deletion of 593 bp from position -122 to +471 in the mutant twisted dwarf1-3 led to a loss of a part of the promoter, the transcription start as well as the first 35 bp of the open reading frame. Size reduction of an *Eco*RI fragment of approx. 600 bp had already been observed in a DNA gel blot experiment. A nucleotide insertion in the third exon at the position +823 and a nucleotide exchange of adenine to guanine at position +829 were identified in the mutant dwarf1-4. The insertion of one nucleotide caused a shift of the open reading frame, causing a translation stop after 85 amino acids . All twisted dwarf alleles are so-called null alleles, which produce no other functional gene product. All of the twisted dwarf mutants studied show the same characteristics of the twisted dwarf phenotype described above.

Example 5:

Identification of the homologs of the *twd* gene from other plant varieties

1. 1. Identification of the twd homolog from *Lycopersicum esculentum*

In order to amplify and subsequently identify homologs of the twd gene from other plants the oligonucleotides twd-S and twd-A were derived as PCR primers from the genetic sequence of the twd gene. By means of these oligonucleotides on the DNA of a cDNA-bank from a tomato (*Lycopersicon esculentum*) sequences of the twd homolog from a tomato were isolated in a PCR under the following conditions:

1 x 94°C 2 min
then 35 cycles:
94°C 1 min
58°C 1 min
72°C 2 min
then 4°C until removal from the PCR machine

Sequence of the primer twd-S
5'-CT(C/T) (G/T)TG C(A/T)T GT(G/T) (G/T)GC TGG GAA TTA G-3'

Sequence of the primer twd-A:
5' -CCA TCC ATT TT(C/T) CTT CT(A/G) T(G/C)T GCT GC-3'

The obtained PCR product (SEQ IC NO:4) was cloned into the vector pGEM-T easy® (Promega) and sequenced by the chain termination method according to Sanger. By means of the sequences of the EST-clones AW038756, AW1895686, AW441601, AW222544 from tomato (*Lycopersicon esculentum*) (GenBank online, Release >115), which was found with the aid of the similarity logarithm BLAST (Basic Local Alignment Search Tool, Altschul et al., Journal of Molecular Biology 215, 403-410 (1990) (tblastn, cutoff for P value: $6e^{-26}$, Matrix: Blosum 62, Gap existence cost: 11, Per residue gap cost:1) with the amino acid sequence of the Arabidopsis TWD protein, a cDNA Contig over

altogether 1142 base pairs could be assembled (TomTWDContig; SEQ ID NO:5). The area of sequence overlaps comprises the nucleotide positions 1 to 95 from TomTWD mit AW441601 and 121 to 140 from TomTWD with AW222544. The translation of the longest open reading frame of the nucleotide sequence of TomTWD Contig in amino acids results in a continuous peptid (TTP) which is 320 amino acids in length. The identity to the TWD protein from Arabidopsis amounts to 74%, the similarity to the amino acid positions 1 to 316 of the TWD protein from Arabidopsis is 85,3%.

2. Identification of the twd homolog from *Zea mays*

By means of the sequences of the EST-clones AW216068 and AW171820 from *Zea mays* (GenBank online, Release > 115), which was found with the aid of the similarity logarithm BLAST (Basic Local Alignment Search Tool, Altschul et al., Journal of Molecular Biology 215, 403-410 (1990) (tblastn, cutoff for P value: $1e^{-31}$, Matrix: Blosum 62, Gap existence cost: 11, Per residue gap cost:1) with the amino acid sequence of the Arabidopsis TWD protein in the non-redundant database of GenBank EST Division/Subdivision *Zea mays*, a cDNA Contig over altogether 776 base pairs could be assembled (ZmTWDContig; SEQ ID NO:7). The translation of the longest open reading frame of the nucleotide sequence of ZmTWD Contig in amino acids results in a continuous peptid (ZmTWD, SEQ ID NO:8) which is 168 amino acids in length. The identity to the TWD protein from Arabidopsis amounts to 68,5%, the similarity to the amino acid positions 196 to 365 of the TWD protein from Arabidopsis is 79,8%.

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INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 7 :		(11) Internationale Veröffentlichungsnummer:	WO 00/50598
C12N 15/29, 15/05, C07K 14/415, C12N 15/82, 5/10	A1	(43) Internationales Veröffentlichungsdatum:	31. August 2000 (31.08.00)

(21) Internationales Aktenzeichen: PCT/DE00/00506

(22) Internationales Anmeldedatum: 18. Februar 2000 (18.02.00)

(30) Prioritätsdaten:
199 07 598.0 22. Februar 1999 (22.02.99) DE

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(81) Bestimmungsstaaten: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO Patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Veröffentlicht
Mit internationalem Recherchenbericht.
Vor Ablauf der für Änderungen der Ansprüche zugelassenen Frist; Veröffentlichung wird wiederholt falls Änderungen eintreffen.

(54) Title: DNA SEQUENCE OF A PROTEIN THAT IS SIMILAR TO FKBP

(54) Bezeichnung: DNA–SEQUENZ EINES FKBP ÄHNLICHEN PROTEINS

(57) Abstract

The present invention relates to a nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 or the fragment or derivative thereof or a nucleic acid sequence which is hybridised with the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7 and which is provided with the biological activity of the nucleic acid sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 or SEQ ID NO:7. The invention also relates to transgenic plants and the seeds thereof, whereby said plants comprise a recombinant inventive nucleic acid sequence.

(57) Zusammenfassung

Die vorliegende Erfindung betrifft eine Nukleinsäuresequenz gemäss SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 oder SEQ ID NO:7 oder deren Fragment oder Derivat oder eine Nukleinsäuresequenz, die mit der Nukleinsäuresequenz gemäss SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 oder SEQ ID NO:7 hybridisiert und die biologische Aktivität der Nukleinsäuresequenz gemäss SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5 oder SEQ ID NO:7 besitzt. Die Erfindung betrifft ferner transgene Pflanzen und deren Samen umfassend eine rekombinante erfindungsgemässe Nukleinsäuresequenz.

Figur 1A

```
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601  TAAATGTTCATCAGTCTATCACCAACAGCTTTCGTATTGCATTGTTCAGTTTGTAGAAAA
661  GGACTTCAAGCTCGCTGATACCGTTATTAGAGGTCTTTTAAAATATTGGCCTGTGACTAA
721  CAGCTCAAAGGAAGTTATGTTTCTTGGAGAGTTAGAAGAAGTCTTGGAAGCAACTCAAGC
781  CGCTGAGTTTCAACGTTGTATGGTTCCATTATCCCGACAAATTGCTCGATGCCTCAACAG
841  TTCACATTTCCAGGTTCGAGTCTTTGACTATCATCACAACTTCATATCTATCTCTCTTGA
901  TAAAGTCTTGTACCTATATATGAAGTTGTACTTTTTGTTTGTCAGGTTGCTGAAAGAGCA
961  TTGTTTCTATGGAACAACGATCACATAAGAAACCTGATCACTCAGAACCATAAAGTGATA
1021 ATGCCTATAGTCTTCCCAGCTCTTGAGAGAAACACGCGTGGACATTGGAACCAAGCAGTT
1081 CAAAGTCTGACTATAAACGTGAGGAAAGTATTATGCGAGATTGACCAAGTTCTTTTCGAC
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1321 ACAGGGTCGTAGTAGGCTCTCGTAGGTTACTATGTACTTGTAACAAATATTTGTGGTCAC
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1441 GTCAAATTGAGAATATTTGATATTATTTTACTGAATTATAATAAACGCCGTTAAAACTCT
1501 CGTTAGTTAACGGCTGACTCTGAAGTGAAAACTGAAAAGTCGAAGGGTCTCTTTATATTT
1561 TCAGAATCAAAATCTGAAATTTATCTCTCGGTCGATCCAGTCTTCGTGAGTGACTTCGAC
1621 GACGACGACGAGTCACACTACTCTTGAGCTTCTCATACTTCGTAAGTTCACTCTCCTCTT
1681 CTCTAAATTGACAAACTTTTTCTTCGTTTTCTGCTATTATTGACGACGAGACTTGATTTT
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Figur 1B

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1801 TTGAATCTTACTTGTCTGATTTGGTCGATGTTTAATCAATTCAACACTTAAAGATTCAAT

1861 TTTTGGATTGACACTTGCACATTTTTATTCAGACCCAGGTTGATTTGGGAAATAATGGAT
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1921 GAATCTCTGGAGCATCAAACTCAAACACATGGTAAGTAAATTTTCATAGATTTAATCTCT
     E  S  L  E  H  Q  T  Q  T  H  D                          13

1981 CTGAATACATATATATGACTTCAATATGTTTGATTGGAGTTTTTTTGTTGTCCCATATTC

2041 AATTGGATGCTTTGTTAAAGGATAAATGTCTATCAAATTATGTTGACTGCGTTATTCTTT

2101 CTAAATCATATTGTGAATCTTGGAACAAAGCATGTATACAACAAATTTGTTAGACTTAAT

2161 AACTCCTTTTCTGTTTGTTAAGAATTGAGAATGACTATTGGGGTTGACTAATGCATCTTT

2221 TGTGGCTCCAGACCAAGAGAGCGAAATAGTTACTGAAGGAAGTGCCGTTGTGCATAGTGA
                   Q  E  S  E  I  V  T  E  G  S  A  V  V  H  S  E  29

2281 GCCATCTCAAGAGGGTAATGTTCCTCCTAAAGTTGATAGTGAAGCTGAGGTCTTGGATGA
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2341 GAAAGTCAGTAAGCAGATTATAAAGGAAGGTCACGGTTCCAAACCATCCAAGTACTCTAC
      K  V  S  K  Q  I  I  K  E  G  H  G  S  K  P  S  K  Y  S  T  69

2401 ATGCTTTTGTAAGTACCCTTTAGCTTTCTGTTGATTGGATGTTGATTTTTCGATTGCACT
      C  F  L                                                    72

2461 TGTTGGCCTATTGCTACTGTTTATTTGAATCTTTCTATCTGACCAATTTCATATTGGCCA

2521 TAGTGCACTACAGGGCATGGACCAAAAACTCGCAGCACAAATTTGAGGATACATGGCATG
           H  Y  R  A  W  T  K  N  S  Q  H  K  F  E  D  T  W  H  E  91

2581 AGCAGCAACCTATTGAATTGGTTCTTGGAAAAGGTATGTGGCTGTCGAATATGTACTCTA
       Q  Q  P  I  E  L  V  L  G  K  E                          102

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2761 CTTCAATGCTGATTAGCCCATTTTAACGTCACTATACAATTTTTTTTATAAAAAAGATTG

2821 TGCACTAAGAGTGAAATGTTGTCTGTGAGACAGAGAAAAAAGAACTAGCCGGTTTAGCCA
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2881 TCGGTGTTGCTAGCATGAAGTCTGGTGAACGTGCGCTTGTGCATGTTGGCTGGGAATTAG
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2941 CTTATGGGAAAGAAGGAAACTTTTCTTTTCCCAATGTTCCACCTATGGCAGACTTGTTAT
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3001 ATGAGGTGGAAGTTATTGGGTTTGATGAAACAAAGGAGGTAAGTTATTTCCTATACCATC
       E  V  E  V  I  G  F  D  E  T  K  E                       163
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Figur 1C

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3121 CTCTCTGACTTAGATGATGTATTGAACAGGGAAAAGCTCGCAGTGATATGACTGTAGAGG
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3181 AAAGGATTGGTGCAGCAGACAGAAGAAAAATGGATGGGAATTCTCTTTTTAAGGAGGAGA
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       L  E  E  A  M  Q  Q  Y  E  M                                204

3301 TCTTTCCAACAATTACGGTCAAAGTTTAGGTTTTCAGGCATACTTAGTGAGTCTGCTCGA

3361 GGCTCTTGTGTCTTCTTTCGGCTTTTGATTAGTCATGGTTTTGCTGTTTCAGGCCATAGC
                                                     A  I  Y  207

3421 ATACATGGGGGACGATTTTATGTTTCAGCTGTATGGGAAGTACCAGGATATGGCTTTAGC
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3481 AGTTAAAAACCCATGCCATCTTAACATAGCAGCTTGCCTCATCAAACTAAAACGATACGA
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3601 ATTAAAGTTCATACTCGGTTTCTCGAAATCTAATCAAACTCAAAACCTTATCAGGTGTTG
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3661 ACAGAAGAAGAGAAAAACCCAAAAGCACTGTTCAGAAGAGGGAAAGCAAAGGCAGAGCTA
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3721 GGACAGATGGACTCAGCACGTGATGATTTCCGAAAGGCACAAAAGTATGCTCCTGACGAC
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3781 AAGGCGATTAGAAGAGAGCTACGAGCACTTGCAGAGCAAGAGAAAGCCTTGTACCAAAAG
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3841 CAGAAAGAAATGTACAAAGGAATATTCAAAGGGAAAGATGAAGGTGGTGCTAAGTCAAAG
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3901 AGCCTTTTTTGGTTGATAGTGTTATGGCAATGGTTTGTTTCCCTTTTCTCCCGTATCTTT
     S  L  F  W  L  I  V  L  W  Q  W  F  V  S  L  F  S  R  I  F  357

3961 CGACGCCACAGAGTTAAAGCAGATTAATGTATGAAGAAGGGTTACAATTA
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351  SLFSRIFRRH RVKAD
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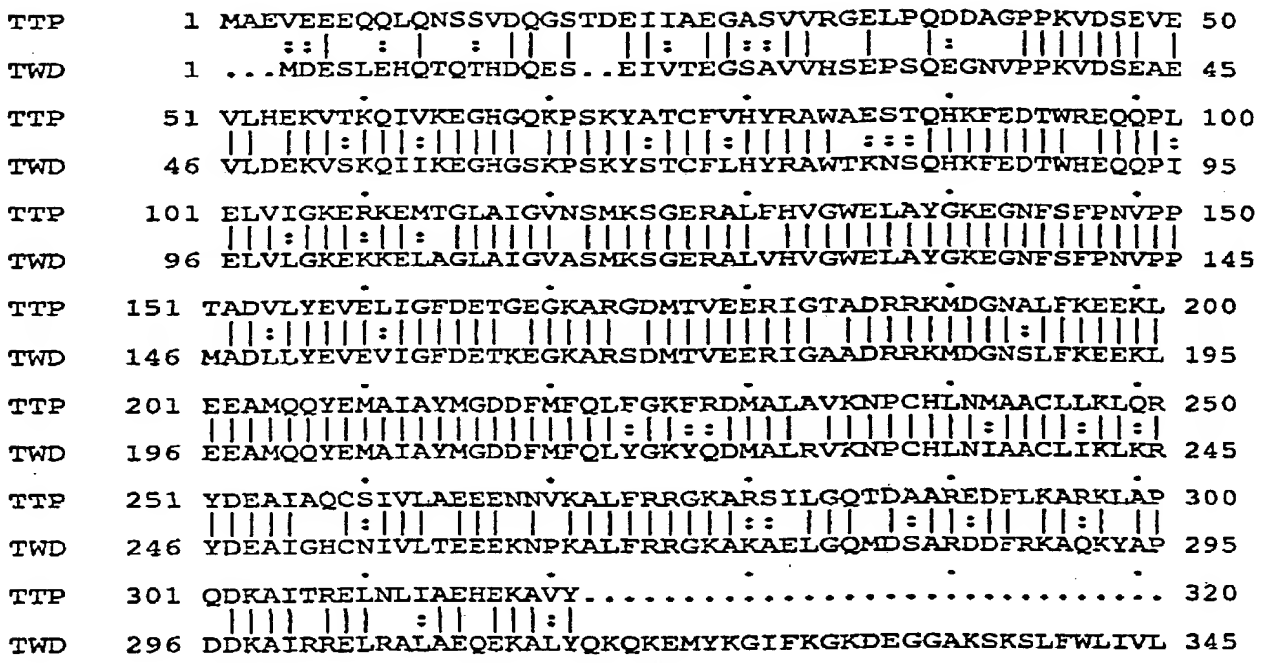
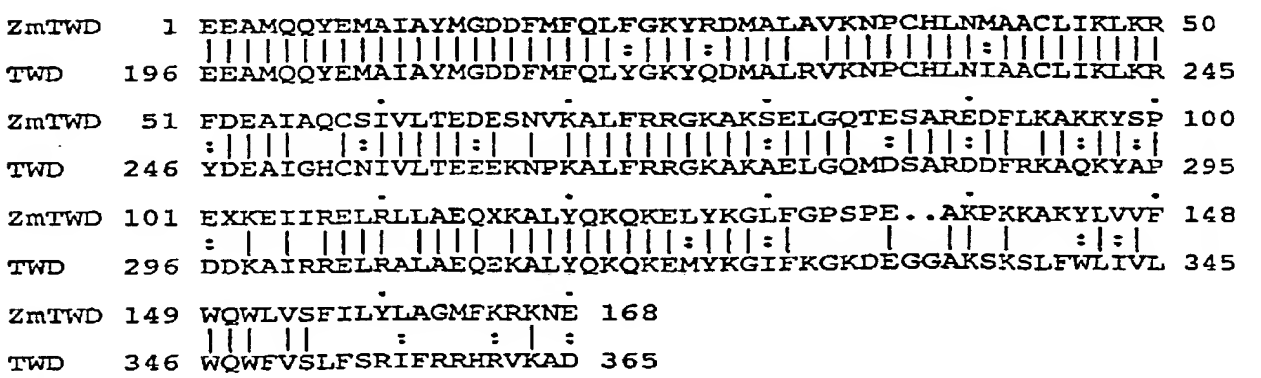
FIGUR 2



FIGUR 3



Docket No.
DEBE1PCTSEQ/dln

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
DNA SEQUENCE OF A PROTEIN THAT IS SIMILAR TO FKBP

the specification of which

(check one)

☒ is attached hereto.

☒ was filed on _____ as United States Application No. or PCT International Application Number **PCT/DE00/00506**

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
DE 199 07 598.0	**Germany**	**22 FEB 1999**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

G. Patrick Sage, #37,710

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25666
PATENT TRADEMARK OFFICE

Direct Telephone Calls to: *(name and telephone number)*
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Sole or first inventor's signature Date 15.12.2000
Residence **Koln, Germany** DEX
Citizenship **German**
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50933 Koln, Germany

Full name of second inventor, if any
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SEQUENCE LISTING

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Asp Glu Ser Asn Val Lys Ala Leu Phe Arg Arg Gly Lys Ala Lys Ser
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Glu Leu Gly Gln Thr Glu Ser Ala Arg Glu Asp Phe Leu Lys Ala Lys
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Lys Tyr Ser Pro Glu Xaa Lys Glu Ile Ile Arg Glu Leu Arg Leu Leu
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Ala Glu Gln Xaa Lys Ala Leu Tyr Gln Lys Gln Lys Glu Leu Tyr Lys
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<212> PRT
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Gly Glu Leu Pro Gln Asp Asp Ala Gly Pro Pro Lys Val Asp Ser Glu
        35                  40                  45

Val Glu Val Leu His Glu Lys Val Thr Lys Gln Ile Val Lys Glu Gly
    50                  55                  60

His Gly Gln Lys Pro Ser Lys Tyr Ala Thr Cys Phe Val His Tyr Arg
65                  70                  75                  80

Ala Trp Ala Glu Ser Thr Gln His Lys Phe Glu Asp Thr Trp Arg Glu
                85                  90                  95

Gln Gln Pro Leu Glu Leu Val Ile Gly Lys Glu Arg Lys Glu Met Thr
            100                 105                 110

Gly Leu Ala Ile Gly Val Asn Ser Met Lys Ser Gly Glu Arg Ala Leu
        115                 120                 125

Phe His Val Gly Trp Glu Leu Ala Tyr Gly Lys Glu Gly Asn Phe Ser
    130                 135                 140

Phe Pro Asn Val Pro Pro Thr Ala Asp Val Leu Tyr Glu Val Glu Leu
145                 150                 155                 160

Ile Gly Phe Asp Glu Thr Gly Glu Gly Lys Ala Arg Gly Asp Met Thr
                165                 170                 175

Val Glu Glu Arg Ile Gly Thr Ala Asp Arg Arg Lys Met Asp Gly Asn
            180                 185                 190

Ala Leu Phe Lys Glu Glu Lys Leu Glu Glu Ala Met Gln Gln Tyr Glu
        195                 200                 205

Met Ala Ile Ala Tyr Met Gly Asp Asp Phe Met Phe Gln Leu Phe Gly
    210                 215                 220

Lys Phe Arg Asp Met Ala Leu Ala Val Lys Asn Pro Cys His Leu Asn
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225 230 235 240

Met Ala Ala Cys Leu Leu Lys Leu Gln Arg Tyr Asp Glu Ala Ile Ala
245 250 255

Gln Cys Ser Ile Val Leu Ala Glu Glu Glu Asn Asn Val Lys Ala Leu
260 265 270

Phe Arg Arg Gly Lys Ala Arg Ser Ile Leu Gly Gln Thr Asp Ala Ala
275 280 285

Arg Glu Asp Phe Leu Lys Ala Arg Lys Leu Ala Pro Gln Asp Lys Ala
290 295 300

Ile Thr Arg Glu Leu Asn Leu Ile Ala Glu His Glu Lys Ala Val Tyr
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atggcataat agtgaacaac atcgtataga gttcataaca actaattgat ccggaccggc 180
cgacagttct acagaaaatt caacactcct tataatacaa ggttggtcaa ttaggccacc 240
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ggtttcgctt caggacttgg cccaaagaga cctttgtaga gctccttctg cttctggtat 420
agggccttgn cttgttccgc gagcaaacgg agctcccgaa tgatctcctt gncttctggg 480
gagtacttct tcgctttgag gaaatcttcc ctcgctgatt ctgtctggcc aagttcagat 540
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ctacactgcg caatagcttc atcgaatctc tttagtttga tcaggcatgc ggccatattg 660
agatggcatg gatttttcac agccaaggcc atgtctctgt actttccaaa taattgaaac 720
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<212> PRT
<213> Zea mays

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            20                  25                  30

Val Lys Asn Pro Cys His Leu Asn Met Ala Ala Cys Leu Ile Lys Leu
        35                  40                  45

Lys Arg Phe Asp Glu Ala Ile Ala Gln Cys Ser Ile Val Leu Thr Glu
    50                  55                  60

Asp Glu Ser Asn Val Lys Ala Leu Phe Arg Arg Gly Lys Ala Lys Ser
65                  70                  75                  80

Glu Leu Gly Gln Thr Glu Ser Ala Arg Glu Asp Phe Leu Lys Ala Lys
                85                  90                  95

Lys Tyr Ser Pro Glu Xaa Lys Glu Ile Ile Arg Glu Leu Arg Leu Leu
            100                 105                 110

Ala Glu Gln Xaa Lys Ala Leu Tyr Gln Lys Gln Lys Glu Leu Tyr Lys
        115                 120                 125

Gly Leu Phe Gly Pro Ser Pro Glu Ala Lys Pro Lys Lys Ala Lys Tyr
    130                 135                 140

Leu Val Val Phe Trp Gln Trp Leu Val Ser Phe Ile Leu Tyr Leu Ala
145                 150                 155                 160

Gly Met Phe Lys Arg Lys Asn Glu
                165
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